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NUTRITION REVIEWS

June 1947

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NUTRITION REVIEWS

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ADEQUATE DIETS FOR ALL PEOPLES—SIZE OF THE TASK

There has been much talk since 1943 about the world's producing food adequate for all mankind. It is high time to get some notions as to the nature and magnitude of the task that the world is setting for itself. Perhaps the following simple analysis of some of the basic data that the FAO and others are now assembling will help toward this end. Table 1 in the FAO's World Food Survey gives estimates of the prewar food consumption of seventy countries in total kilograms per head per year, and these broken down into eight food groups: cereals, roots and tubers, sugar, fats, pulses (dried beans and peas), fruits and vegetables, meat, milk, and scattering estimates on wine and beer. Table 2 converts these to calories per day and to grams of protein per day.

The best way to sense these figures is to compare them with food consumption of the United States in the same 1935–39 prewar years. This can be done approximately by using the same method that is used here in the United States for comparing the changes in our food consumption from year to year. The press, from time to time during these last few months, has emphasized that per capita food consumption in the United States is now 16 per cent higher than before the war. How was this 16 per cent figured? Simply by multiplying the average per capita amounts of each food consumed in 1946 by the prices in 1935–39, doing the same in the base period 1935–39, and dividing the first of these by the second. The result thus obtained for 1946 was 116, or 16 per cent above 100 for 1935–39. This rise of 16 per cent has come about very little because more calories of food are eaten now than before the war—calories were up only 100 from 3250; but because consumption has shifted toward more of the expensive types of food, toward more dairy products, poultry products, meats, fruits, and vegetables. The index number for 1945 comparable with 116 for 1946 was 112; for 1942, 107; for 1939, 104; for 1935, 96.

Observe how some of the prewar diets of other countries compare with the prewar diet of the United States when expressed in terms of index numbers figured in this same way. Only three were higher, New Zealand at 113, Argentina at 104, and Australia at 106. Denmark's was 97, and the United Kingdom's was 88; France's, 84; Russia's, 59; Italy's, 56. In South America, Brazil's was only 76; Chile's 60; and Peru's, 39. In Central America, Cuba's stood at 63; but Puerto Rico's at only 47; and Mexico's at 48. The lowest were in densely populated Asia, with India and Java at 33, and China at 35.

The estimates for total calories in the World Food Survey, when similarly reduced to index numbers, range from 101 for New Zealand, 97 for Argentina, and 92 for the United Kingdom, and 87 for Russia, down to 70 for Japan, 68 for China, 62 for India, and 59 for Korea and Mexico. Actual ingestion of food does not vary this much. The estimates are necessarily based on food in the market place, at retail levels, and wastes and losses are much greater in some countries than in others. Nevertheless, this index indicates, except for inaccuracies and omissions in the records, differences in food "available for consumption." The index numbers for composition of the diet range from 51 for China to 112 for New Zealand, with countries like Russia about halfway between.

The World Food Survey, after presenting the forementioned estimates of prewar food

consumption by countries, then proceeded to work out food "targets" or goals for these countries. These were not intended as ultimate goals, but merely as something practical to aim at in the first stages of a program of attaining adequate food for all peoples. With no increase in the population, these targets call for the changes in total food production in the first column following. These targets are based roughly on a minimum of 2600 calories, and an adequate diet at moderate cost in each country. Given an increase of 25 per cent in the world population between 1935 and 1960, as assumed to be probable in this Survey, the amounts required would be as in the second column following.

	Targets only	*Targets plus 25 per cent population increase*
Cereals	−3	+21
Roots and tubers	+1	+27
Sugar	−10	+12
Fats	+7	+34
Pulses	+44	+80
Fruits and vegetables	+110	+163
Meat	+17	+46
Milk	+60	+100

The 1935–39 index of world food consumption, computed as in the foregoing, with the latest reported populations of the countries used as weights, was 53, consisting of 65 for composition of the diet, and 81 for calories. The target set in the first column would raise it to 65, divided 75 and 87 respectively.

The targets for only a few of the separate countries are published in the Survey. To obtain the one set for the United States would raise its index of food consumption to 110. The one set for the United Kingdom would raise it from the present 88 to 103; that for India, from its present 33 to 55; that for China, from its present 35 to 55. The increase from 88 to 103 proposed for the United Kingdom would raise the calory level only from 92 to 93, but the composition level from 96 to 112. The increase proposed for India would raise its calory level from 62 to 80 and its composition level from 53 to 69.

What are the possibilities of producing these food increases in the world, especially the increases in the foods needed to attain the composition indexes? These are foods that require much land and labor per unit of product. Dr. R. M. Salter of the Department of Agriculture, in a recent paper before the American Association for the Advancement of Sciences, estimated that the known technologic advances applied to land now in crops and pasture could supply the 1960 targets only for roots and tubers and sugar. He then estimated that around a billion more acres of red tropical soils, and 300,000 acres of cold northern soils, could be improved and brought into use, although at great expense. Even if all of these were developed, we would not have enough land to produce the meat and milk called for by the 1960 targets. And if the world's population were to continue to increase at the rate of 1 per cent a year, before the year 2000 there would be land enough left to feed its people on little more than on diets of cereals, roots, and tubers.

Fortunately for the world, population history does not develop in this way. In nearly half the world, diets are already gaining faster than the population. Other countries will fall into this progression one after another, and ultimately China and India. Nevertheless, the world has set a large task for itself in raising food consumption to an adequacy level; and not the least of the problems connected therewith is population growth in India, China, much of South and Central America, and many other parts of the world.

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PRESENT KNOWLEDGE OF NIACIN IN NUTRITION

The discovery of niacin as the antipellagra vitamin was announced by C. A. Elvehjem, R. J. Madden, F. M. Strong, and D. W. Woolley in 1937 (*J. Am. Chem. Soc.* **59,** *1767 (1937)*) following experiments upon the canine disease blacktongue, which had been established as the analogue of human pellagra by the work of Goldberger and his colleagues (J. Goldberger and G. A. Wheeler, *Pub. Health Reports* **43,** *172 (1928)*) During the following year a number of clinical trials were made which established beyond doubt the value of niacin in the treatment of pellagra, and that the acid or its amide should be regarded as the P-P (pellagra-preventive) factor.

This discovery did not, however, resolve all the problems concerned with either canine blacktongue or human pellagra. There is no clear idea of the way in which the symptoms and signs of the disease follow upon simple deprivation of niacin. Indeed, it has been found that blacktongue is not necessarily produced by depriving the animal of niacin alone, and that it may be cured by the injection of saline alone (*Nutrition Reviews* **3,** *267 (1945)*). In the treatment of human pellagra it was early recognized that although the mental symptoms, glossitis, and alimentary symptoms respond rapidly to niacin therapy, the peripheral neuritis and the skin and ocular symptoms commonly associated with pellagra require thiamine and riboflavin respectively for successful cure. Thus it appears that human pellagra is a mixed deficiency, and more recently it appears that the anemia occurring in blacktongue is due primarily to a simultaneous folic acid deficiency (W. A. Krehl, N. Torbet, J. de la Huerga, and C. A. Elvehjem, *Arch. Biochem.* **11,** *363 (1946)*).

Over and above the mixed vitamin deficiency nature of blacktongue and pellagra, it has been evident, although temporarily overshadowed by the discovery of niacin as the antipellagra factor, that some other factor is involved. The role of corn in the etiology of pellagra has been discussed (*Nutrition Reviews* **3,** *267 (1945)*; **4,** *334 (1946)*), and it is pertinent to note that the dog develops blacktongue more uniformly and rapidly on the Goldberger diet, which contains corn meal, than on a purified diet. The pellagragenic action of corn has been attributed both to the lack of tryptophane in corn protein and to the presence of a positive toxic agent in this cereal grain. In support of the former theory, W. A. Krehl and co-workers (see *Nutrition Reviews* **4,** *182 (1946)* demonstrated that more niacin is required by the rat as the tryptophane content of the diet is decreased. There is evidence that tryptophane is actually a precursor of niacin (W. A. Perlzweig, F. Rosen, N. Levitas, and J. Robinson, *J. Biol. Chem.* **167,** *511 (1947)*); H. P. Sarett and G. A. Goldsmith, *Ibid.* **167,** *293 (1947)*). The recognized value of milk in the treatment of pellagra, despite a rather low content of niacin, is possibly explained on the basis of its tryptophane content as well as other B-vitamins.

In support of the toxic theory, D. W. Woolley (*J. Biol. Chem.* **163,** *773 (1946)*) and E. Kodicek, K. J. Carpenter, and L. J. Harris (*Lancet* **II,** *491 (1946)*) claim the existence of a growth-depressing factor in maize. The latter workers identify the factor as indole-3-acetic acid. Confirmation of these reports is awaited, but at this time it appears possible that both the tryptophane deficiency and toxic agent factors are operative in the pellagragenic action of corn.

The recommended dietary allowance for niacin is from 12 mg. daily for man on 2500 calories to 20 mg. on 4500 calories, but it is evident that this figure must depend on the types of food in the diet. Furthermore, there is evidence that niacin may be synthesized in the intestine and utilized (see

Nutrition Reviews **4,** *76* (*1946*)). In general, meats and poultry constitute the important human food sources of niacin, although yeast and peanuts are rich sources. Cereals contribute a significant amount, while fruits and vegetables are regarded as poor sources (*Ibid.* **4,** *233* (*1946*)). In addition there appears to be a niacin precursor in some foods which escapes analysis by frequently used procedures (*Ibid.* **3,** *120* (*1945*)). Niacin in foods may be determined by variations of the cyanogen bromide reaction, by microbiologic means (W. A. Krehl, F. M. Strong, and C. A. Elvehjem, *Ind. Eng. Chem.* (*Anal. Ed.*) **15,** *471* (*1943*)), and by the human bioassay technic of D. Melnick and co-workers (*Nutrition Reviews* **4,** *74* (*1946*)).

Earlier studies of the metabolism of niacin suggested that nicotinuric acid and the methylated compound trigonelline were the two compounds derived from niacin which appear in human urine (W. A. Perlzweig, E. D. Levy, and H. P. Sarett, *J. Biol. Chem.* **136,** *729* (*1940*)), and it was established that the extra excretion of acid hydrolyzable niacin and trigonelline after a test dose of niacinamide may serve as an indication of the nutritional status of an individual with respect to niacin. To make such an evaluation, however, it is necessary that the subject shall be on a rigidly controlled trigonelline-free diet. Later it was established that the chief methylated end product in the urine is N′-methylnicotinamide, and that after a large dose of niacinamide as much as 20 to 30 per cent may be excreted in this form. It has been suggested that human urine also contains a more completely oxidized product, namely N′-methyl-6-pyridone-3-carboxylamide, in an amount which may be equal to that of N′-methylnicotinamide (W. E. Knox and W. I. Grossman, *Ibid.* **166,** *391* (*1946*). Some of the excretion forms of niacin may be estimated separately (*Nutrition Reviews* **4,** *58* (*1946*)), but the use of these analyses for determining the adequacy of niacin nutrition is still not a sound basis.

Despite claims for other functions of niacin, it appears that its principal role is as a constituent of the coenzymes I (diphosphopyridine nucleotide) and II (triphosphopyridine nucleotide). These coenzymes are essential in biologic oxidation, especially in the oxidation of carbohydrate. These catalysts are reduced in amount in the tissues of blacktongue dogs. There is evidence, as yet unconfirmed, that a marked decrease in coenzyme I occurs in the heart muscle under anoxic conditions (*Nutrition Reviews* **3,** *236* (*1945*)).

Advantages of niacinamide over niacin for therapeutic use have been reviewed (*Nutrition Reviews* **4,** *65* (*1946*)), and a pharmacologic application of niacin for angina has been suggested (F. J. Neuwahl, *Lancet* **II,** *419* (*1942*)).

VITAMIN E IN HEART DISEASE

A goal which naturally intrigues clinicians is to find clinical uses for substances which have clearcut roles in animal nutrition. A number of the vitamins have yet to find their place in relation to human disease; among these are pyridoxine, pantothenic acid, and vitamin E. Early attempts to demonstrate a role for vitamin E in preventing abortions in women failed; nor does this vitamin seem to have anything to do with sterility in the male. A place for vitamin E in relation to muscular dystrophy in man has yet to be demonstrated; A. Vogelsang, E. Shute, and W. Shute (*Med. Record* **160,** *21* (*1947*)) attempt to give it a place in the management of heart disease.

A. Vogelsang and E. Shute (*Nature* **157,** *772* (*1946*)) in a preliminary report pointed out that while studying the effects of high dosage of vitamin E on purpura, the good

influence of this factor upon coronary heart disease became apparent. In a more recent report 10 case histories are given in which the administration of vitamin E in large doses (200 to 600 mg. of Ephynal) is described. Administration of this amount of vitamin E had no apparent effect upon normal hearts even after administration for many months. Its effect upon patients having congestive heart disease and the anginal syndrome, on the other hand, is said to be marked. It increases exercise tolerance, diminishes or abolishes anginal pain during the period of its administration, and has a pronounced diuretic effect. The effect of vitamin E upon coronary pain is attributed to a direct action on the coronary vessels or to some action on the metabolism of the heart muscle. The first possibility is based on a report by E. Shute (*J. Obstet. Gynec. Brit. Emp.* **49,** *534 (1942)*) in which this vitamin was claimed to cause dilatation of the local capillaries in senile vulvitis. The observations of these investigators on purpuras raise another possibility, namely, that small hemorrhages into the walls of the coronaries or into the heart muscle itself may produce pain. Administration of vitamin E is alleged to prevent such extravasations or causes them to be absorbed.

It is difficult to evaluate a report such as this but one cannot restrain a feeling that the account is distinctly premature and unwarranted. The statement in *Nature* consists of three paragraphs and the longer account in the *Medical Record* offers nothing to suggest that an objective study was made. Any clinician familiar with the course of patients with heart disease, particularly coronary disease, knows that one cannot draw conclusions from the study of 10 cases. These cases included hypertension, angina pectoris, auricular fibrillation, rheumatic fever with valvular lesions and with failure, and coronary thrombosis. The influence of time, rest, and psychic factors receives no mention in the account and improvement in exercise tolerance is claimed without any indication as to how this was measured. How cautiously experts in the field of heart disease approach the problem of the effect of various agents for the treatment of coronary thrombosis, is indicated by the means whereby the use of Dicumarol in the management of coronary thrombosis is being evaluated; a number of investigators throughout the country are studying a large series of patients using alternate cases as controls.

The quality of the study by Vogelsang and his associates may perhaps be judged by comments in regard to one of the cases. This patient complained of occasional dizzy spells and had a moderate anemia for which iron was given. Since iron, it is stated, "seems to counteract the effect of vitamin E," and would therefore be contraindicated, the patient was given, instead, 15 units of liver extract intramuscularly. After the first two injections, the dizzy spells disappeared. It is implied that the vitamin E was no longer being "counteracted."

EFFECT OF PREOPERATIVE FORCED FEEDING ON SURGICAL PATIENTS

An earlier review (*Nutrition Reviews* **4,** *6 (1946)*) pointed out that tissue injury is associated with nitrogen losses which may be large. J. E. Rhoads and associates have observed that patients recovering from gastric and intracranial operations are generally in negative nitrogen balance during the first five days after operation and tend to regain positive balances in the second five days in most instances. The significance of such a negative nitrogen balance is not clear nor is it known what influence it may have on the convalescence of the patient. In any event, it would seem desirable to overcome this negative balance. Unfortunately, however, the problem of

feeding certain groups of patients during the first five days postoperatively is a difficult one, often requiring special technics such as intravenous or orojejunal feedings. C. E. Koop, J. H. Drew, C. Riegel, and J. E. Rhoads (*Ann. Surgery* **124**, *1165 (1946)*) therefore attempted to induce a large enough positive nitrogen balance by a vigorous feeding program before operation in those patients who could take food by mouth readily before operation, thus avoiding the difficulty of maintaining nitrogen balance in the early postoperative period under trying and difficult circumstances.

Eleven patients were studied carefully pre- and postoperatively and were "force-fed." These included 2 cases of gastric resection, 2 in which a part of the large bowel was removed, and 7 in which brain operations were performed. The attempt was made to feed the patients a diet preoperatively which would provide them with three times the nitrogen which had been usually found to be adequate to produce postoperative equilibrium. In all instances it was possible to induce a nitrogen intake above 0.8 g. per kilogram per day. Three palatable meals were provided most patients each day and additional protein was supplied as hydrolysates of casein or lactalbumin at intervals while the patients were awake. Postoperatively 4 of these patients were given 0.23 g. of nitrogen per kilogram of body weight per day or more and 30 calories per kilogram per day, while 7 patients were given the usual hospital regimen of intravenous glucose, food being supplied on the fourth or fifth day.

The "controls" consisted of 12 patients: 5 on whom gastric resection had been performed, 2 on whom part of the large bowel had been removed, and 5 on whom brain operations had been carried out. This control series was not studied during the five days before operation as were the subjects described above and for this reason these patients were not controls in the strictest sense. It is assumed that these patients had either maintained themselves in equilibrium or were in negative balance during the week prior to operation. Postoperatively the control subjects were given glucose intravenously as well as the hospital diet or protein hydrolysate by vein. Four patients received intakes which should have been sufficient to achieve nitrogen equilibrium. Postoperatively, the daily average nitrogen intake in the force-fed group ranged from 0.01 to 0.28 g. per kilogram and in the control group it was 0.01 to 0.52 g. per kilogram per day.

In the force-fed group large positive balances were accumulated in every case during the five preoperative days. The extremes were 57.6 to 148.0 g. for the five day period, and the mean was 92.2 g. or an average of 18.4 g. positive nitrogen balance per day. Thus the daily gain was greater than the nitrogen content of one-half pound of fresh muscle.

When the postoperative negative period was balanced against the preoperative positive balance, in 10 of the 11 patients there was a net positive balance. The balances ranged from a minimum of −3.1 to a maximum of +113.3 g. with a mean of 55.2 g. for the ten day period for the 11 patients. Preoperatively in the force-fed group, 6 of the 11 patients sustained a fall in serum protein concentration while 5 remained the same or showed an increase. However, all changes but one were minor, and one of the 2 who showed a rise in serum protein concentration had an initial level below 7.5 g. per 100 ml. Postoperatively there was little difference between force-fed and control groups. By making plasma volume determinations it was possible to calculate the total circulating plasma protein. Only one of the 8 force-fed patients had more circulating plasma protein at the end of the ten day study than at the beginning of the feeding regimen. It is of interest that this patient was the only individual among the 8 whose initial serum protein concentration was below 6.0 g. per 100 ml.

However, both before and after operation the differences were small. The data suggest that patients with normal serum protein levels do not store appreciable amounts of protein on the force-feeding regimen employed.

Interesting data were obtained concerning the physical status of the subjects. Because clinical impressions are so apt to be misleading, objective means of measuring possible benefits were used. These included measurement of the response of the patient's circulation to changes in position on a tilt table equipped with a foot board. In addition, the status of the circulation including the cardiac output was studied by means of a portable ballistocardiograph. There are many variables in the reaction of pulse and blood pressure to change in position from the horizontal to 15° from the vertical on a tilt table, but the data obtained are of interest, nevertheless. Both the force-fed and control subjects acted essentially like normal individuals before operation when subjected to changes in position on the tilt table. Of the 8 patients in the force-fed group who were examined postoperatively, one fainted and one who was about to faint was returned to the horizontal position because of the intensity of his subjective complaint. Of 6 subjects in the control group, 3 fainted. The average drop in systolic blood pressure was almost identical in the two groups. Pulse rate elevations, however, were dissimilar. In the force-fed group the average rise in pulse was 22 beats per minute while in the control group the rise was 32 beats per minute.

Differences between the two groups were brought out more clearly when paired experiments were studied. In selecting pairs attention was given to operative procedure and age group. In this way 3 general surgical and 3 neurosurgical pairs were matched. Three of the paired controls lost consciousness with change of position to the vertical while none of the force-fed partners fainted. Other changes such as absent radial pulses, excessive sweating, and dizziness in the vertical position were much more evident in the control than in the force-fed group.

Again, ballistocardiographic abnormalities occurred much more frequently after operation in all the patients whose nitrogen balances were negative than in the force-fed group. From paired comparison it was apparent that the individual in better metabolic condition developed fewer ballistic abnormalities after operation than did the control patient with whom he was paired. The results were not significant statistically but the chances were calculated as being 92 out of 100 that a negative nitrogen balance is associated with the development of ballistocardiographic abnormalities after operation.

In addition to these objective observations marked differences in the clinical status of the two groups were noted. Those who had been force-fed needed ventricular taps for increased intracranial pressure less frequently than did controls. They were ambulatory sooner and showed less postoperative facial edema. Results were more difficult to measure in the general surgical group but all the force-fed patients had benign postoperative courses despite several of them having had extensive unavoidable traumatic and time-consuming procedures.

The investigators concluded that in those patients who can be fed by mouth or by tube, it seems logical to spend five days before operation with a vigorous nutritional program at a time when very high nitrogen and caloric intakes are possible and when the patient is in comparative comfort rather than to spend the same number of days postoperatively in attempting to achieve nitrogen equilibrium under trying and difficult circumstances.

CONJUGATED FORMS OF FOLIC ACID IN TREATMENT OF SPRUE

When folic acid (pteroylglutamic acid) is given orally or parenterally (*Nutrition Reviews* **4,** *102 (1946)*) it is effective in relieving the symptoms and the macrocytic anemia associated with pernicious anemia, sprue, and related forms of macrocytic anemia. However, the relationship of this interesting observation to the pathogenesis of pernicious anemia and the related disorders is by no means clear. Castle postulated that there exists in pernicious anemia a deficiency of "intrinsic factor," a secretion of the gastric mucosa. Through lack of "intrinsic factor" the patient fails to obtain from an "extrinsic factor" contained in the diet a substance or substances necessary for the formation of an "erythrocyte maturing factor" present in liver extract which is capable of relieving the manifestations of this disease.

Since folic acid occurs in foods, one would expect to link it to the "extrinsic factor" of Castle. However, the fact that folic acid is effective when given parenterally throws doubt on such an hypothesis although it must be admitted that the extrinsic factor as defined by Castle, could not be given parenterally since it has never been purified and therefore its activity by the parenteral route is unknown.

Folic acid occurs naturally as the free vitamin and also in the form of various complexes or conjugates. Two of these complexes, both polyglutamates, have been isolated in pure form, one from yeast and one from a filtrate obtained from an aerobic fermentation of a diphtheroid-type organism. The folic acid complex of yeast, often termed vitamin Bc conjugate, was identified by J. J. Pfiffner, D. G. Calkins, E. S. Bloom, and B. L. O'Dell (*J. Am. Chem. Soc.* **68,** *1392 (1946)*) as pteroylhexaglutamylglutamic acid. The other naturally occurring complex, usually termed fermentation folic acid or fermentation Lactobacillus casei factor, was described by B. L. Hutchings, E. L. R. Stokstad, N. Bohonos, and N. H. Slobodkin (*Science* **99,** *371 (1944)*) and was shown to be a triglutamate (pteroyldiglutamylglutamic acid). Fermentation folic acid appears to be utilized effectively by L. casei, exerting an effect deviating from that of crystalline pteroylglutamic acid only as the molecular weights differ. For Streptococcus faecalis the compound has only about one-fiftieth the activity of pteroylglutamic acid. The conjugate of yeast (heptaglutamate) is essentially unable to stimulate the growth of either microorganism. In animal species known to require folic acid such as the monkey, chick, and rat each of the conjugates exerts an effect essentially equivalent to its folic acid content. It thus seems probable that the activity of these compounds results from their serving as a source of free folic acid. This is split off by the action of "vitamin Bc conjugase."

Some patients with pernicious anemia seem to be unable to release folic acid from certain preparations of heptaglutamate while in other patients this biochemical defect has not been present. Thus, A. D. Welch, R. W. Heinle, E. M. Nelson, and H. V. Nelson (*J. Biol. Chem.* **164,** *787 (1946)*) and F. H. Bethell and co-workers (*Univ. Hosp. Bull., Ann Arbor* **12,** *42 (1946)*) reported that the conjugated folic acid in yeast was little or not at all utilized by certain patients with pernicious anemia whereas normal human subjects demonstrated their ability to derive folic acid from the same lots of this heptaglutamate by markedly increasing their urinary excretion of the free vitamin following the oral or parenteral administration of the conjugate. On the other hand, others have observed patients with pernicious anemia who were able to utilize concentrates of the yeast form of conjugated folic acid. This could mean that there exist etiologic differences in the disease which heretofore

have not been appreciated or that there simply exist quantitative differences in the degree of deficiency of "intrinsic factor" or of "erythrocyte maturation factor." The situation becomes more complicated, however, when one considers the observation that one or more substances occur naturally, for example in yeast, that are capable of competitively inhibiting some of the conjugases which release folic acid from pteroylhexaglutamylglutamic acid. The success or failure of treatment of cases of pernicious anemia with folic acid conjugates may possibly be attributable, therefore, to differences in the various concentrates of vitamin Bc conjugate rather than differences in the patients. R. M. Suárez and co-workers (*J. Lab. Clin. Med.* **31,** *1294 (1946)*) suggest that one of the defects in patients with pernicious anemia may be found to consist of a partial to complete inability to inactivate such inhibitors of conjugases.

T. D. Spies (*South. Med. J.* **39,** *634 (1946)*) reported that fermentation folic acid in a daily intramuscular dose of 3 mg. given during a period of eleven days caused a submaximum reticulocyte response in a patient with pernicious anemia in relapse. In the paper cited above, Suárez and associates report on the effect of folic acid complexes in the treatment of 2 cases of tropical sprue.

Both cases were classic examples of tropical sprue and both had macrocytic anemia of severe degree. After adequate control periods and under suitable dietary restrictions the first patient was given fermentation folic acid by daily intramuscular injection. The material injected was shown by microbiologic assay to contain approximately 4.9 mg. of fermentation folic acid in each daily dose, equivalent to about 3.1 mg. of pteroylglutamic acid. Three days after initiation of treatment with this substance, pronounced clinical improvement took place. On the fourth day of treatment the reticulocyte level had reached a peak of 18 per cent and this level was maintained until the tenth day of treatment when the daily intramuscular administration of 1 ml. of liver extract (15 units) was begun in addition to the fermentation folic acid. By this time the erythrocyte count had increased but the number of leucocytes and of platelets had shown little change. Following the injections of liver extract the number of white blood cells and platelets increased and there was a persistence of the reticulocytosis together with a progressive increase in the number of erythrocytes and in the amount of hemoglobin.

Studies of the excretion of folic acid in the urine in this patient showed that prior to treatment amounts of the free vitamin were found which were strikingly lower than those usually encountered in normal subjects or in those with pernicious anemia. Administration of the conjugate was associated with the elimination in the urine of the free folic acid in an amount indicating that a large proportion of the conjugate was converted *in vivo* to the free vitamin. When purified liver extract free of folic acid was administered in addition to fermentation folic acid, a much greater increase in the urinary excretion of free folic acid occurred, a circumstance which suggests that the erythrocyte maturation factor present in liver extract may be concerned with the release of stored complexes of folic acid or that in some other manner it affects the metabolism of folic acid.

The second patient was given a concentrate of vitamin Bc conjugate of yeast by mouth in daily doses equivalent to 8.4 mg. of free folic acid. This was associated with rapid clinical improvement and the reticulocyte response which was observed corresponded to the maximal effect expected in such a patient following liver extract therapy. Urinary studies showed at first only small increases in the excretion of the free vitamin but the amount rose gradually as treatment was continued. As in the first case, the injection of purified liver

extract free of folic acid was followed by a still further rise in the urinary excretion of folic acid.

These observations are of considerable interest but it must be admitted that as yet the discovery of the effectiveness of folic acid and its conjugates in the treatment of pernicious and related anemias has not simplified the question of the pathogenesis of these anemias. Elucidation of the new problems which have arisen is delayed by limitations in the amounts of folic acid complexes available for testing in patients and by scarcity of cases in which tests can be made. At the present time a plausible thesis is that one of the biochemical defects resulting from deficiency of the erythrocyte maturating factor is an abnormality in the derivation of folic acid from its conjugates. This abnormality may lie in the mechanism through which the body copes with inhibitors of the enzymes responsible for the release of the vitamin from its naturally occurring complexes. A defect of this type Welch, Suárez, and their associates argue, could be either partial or complete, possibly reflecting the fact that variable degrees of deficiency of intrinsic factor and probably of erythrocyte maturation factor are encountered.

THE DEPENDENCE OF THE VITAMIN B_6 CONTENT OF MOUSE TISSUE ON DIETARY PROTEIN

During the last few years it has become clear that vitamin B_6 is involved in the metabolism of the amino acids (*Nutrition Reviews* **4,** *150, 163, 232 (1946)*). E. C. Miller and C. A. Baumann (*J. Biol. Chem.* **157,** *551 (1945)*) showed that mice deprived of vitamin B_6 have a higher mortality rate on high protein diets than when the protein content of the diet is moderate, and that the amount of vitamin required for a given growth response rises as the consumption of protein is increased. These observations suggest the question of whether the consumption of protein exerts an adverse effect upon the concentration of vitamin B_6 within the animal, a question which has been put to the experimental test by B. S. Schweigert, H. E. Sauberlich, C. A. Elvehjem, and C. A. Baumann (*Ibid.* **165,** *187 (1946)*).

The method of attack on this problem was to feed the experimental animals one of two complete simplified diets, containing 10 and 50 per cent of casein respectively, with or without additions of pyridoxine, and after varying lengths of time to kill the animals and assay the pyridoxine content of those tissues (chiefly liver) chosen for analysis by a microbiologic method. The livers of weanling mice contained on the average 9.8 micrograms of vitamin B_6 per gram of tissue. When vitamin B_6 was omitted from the diet this level in the liver tissue decreased whether the amount of casein in the diet was high or low, but the rate of decrease was much faster on the high protein intake.

When the mice were placed on diets deficient in vitamin B_6, the amounts of hepatic vitamin B_6 depended among other things on the size of the mice at the beginning of the depletion period. This effect was not observed on the 10 per cent casein diet but was established with the 50 per cent casein diet. The faster rate of depletion of tissue vitamin B_6 did not appear to be due to an increased loss of vitamin in the urine, since similar amounts of vitamin were found in the urine from the low and high casein groups. On the other hand, the lower amounts of vitamin B_6 in the liver were not due to a reduction of food intake cn the higher level of casein.

To observe the effect of casein on storage of vitamin B_6 young mice were fed either the high or the low protein diet with amounts of pyridoxine ranging from 0 to 1.0 mg. per 100 g. of diet. The diets were fed for three to four weeks before the mice were killed for determination of hepatic vitamin

B_6. The mice deprived of pyridoxine lost weight on the 50 per cent casein diet, while those on the 10 per cent casein diet gained slightly. Optimal growth occurred on the low protein diet when it contained 0.05 microgram of pyridoxine per 100 g., but on the high protein diet 0.25 microgram of pyridoxine per 100 g. was required to cause an optimal growth response. Corresponding with this, the hepatic storage of the vitamin on low levels of pyridoxine intake was slightly lower on the 50 per cent than on the 10 per cent casein diet. On the other hand, at higher levels of pyridoxine intake, on diets containing 0.5 to 1.0 microgram per 100 g. of diet, the high protein diet appeared to promote the storage of the vitamin, a curious reversal of the effects of the low and high protein diets for which there does not seem to be any satisfactory explanation.

Observations were also made on other proteins and amino acids. Comparison was made of the storage of vitamin B_6 on diets devoid of vitamin B_6 and containing as protein source either 10 per cent casein, 20 per cent casein, 20 per cent fibrin, 20 per cent egg albumin, 20 per cent lactalbumin, 50 per cent casein, 10 per cent casein plus 0.96 per cent dl-tryptophane, and 10 per cent casein plus 16 per cent of a mixture of amino acids. Twenty per cent fibrin was fed because it contains as much tryptophane as 50 per cent casein. The 0.96 per cent tryptophane was calculated to contain the same amount of l-tryptophane as the "extra" 40 per cent of casein; similarly the amounts of l-alanine, l-phenylalanine, l-tyrosine, l-asparagine, and l-glutamic acid in the mixture of amino acids contained the l isomers in the same amounts as in casein.

The mice on 50 per cent of casein lost an average of 0.4 g. weekly for four weeks, and at the end of this time their livers contained less vitamin B_6 than those which received 10 per cent of casein and gained 0.7 g. per week. On all the other protein sources the mice grew at least as well as on 10 per cent casein, and at the end of the experiment their livers contained as much vitamin B_6. It was thus demonstrated that tryptophane was not the main factor responsible for depletion of the vitamin from the liver of mice on high protein diets.

It was interesting that many of the mice on diets low in vitamin B_6 developed fatty livers, from which as much as 15 per cent of the fresh weight was extractable with ether. This condition appeared much more rapidly on the high casein than on the low casein diet, and in general seemed to parallel the other evidences of severe vitamin B_6 deficiency such as loss in weight and low concentration of the vitamin in the tissues. Addition of tryptophane to the diet either as dl-tryptophane or as fibrin seemed to hasten the appearance of fatty livers.

There was a species difference between the rat and the mouse. Weanling rats were treated in the same way as the mice described above, and the observations showed that the high protein diet did not hasten the depletion of vitamin B_6 in the liver, nor was the storage of the vitamin affected at a comparatively high level of pyridoxine intake. This accords with the earlier observations of E. C. Miller and C. A. Baumann (*loc. cit.*); *J. Biol. Chem.* **159,** *173 (1945)*) that the survival of the pyridoxine deficient rat is affected only to a negligible extent by an increase in dietary casein level.

ESSENTIAL FATTY ACIDS IN RATS

In 1929 G. O. Burr and M. M. Burr (*J. Biol. Chem.* **82,** *345 (1929)*) showed that rats fed completely fat free diets developed symptoms which eventually led

to death but which could be prevented or cured by administration of linoleic acid or of fats which contained this doubly unsaturated acid. Although their diets were also deficient in certain B-vitamins which were unrecognized at that time, the essential nature of linoleic acid, or other highly unsaturated fatty acids such as arachidonic acid, has been amply confirmed with complete diets (F. W. Quackenbush, H. Steenbock, F. A. Kummerow, and B. R. Platz, *J. Nutrition* **24,** *225* (*1942*); F. W. Quackenbush, F. A. Kummerow, and H. Steenbock, *Ibid.* **24,** *213* (*1942*)). Deficiency symptoms following insufficient administration of essential fatty acids have also been noted in mice (E. A. White, J. R. Foy, and L. R. Cerecedo, *Abstracts, Am. Chem. Soc., 103rd Meeting, Memphis, Tenn.* (*Div. Biol. Chem.*) *p. 7* (*1942*)) and dogs (A. E. Hansen and H. F. Wiese, *Proc. Soc. Exp. Biol. Med.* **52,** *205* (*1943*)).

The inability of mice and rats to synthesize these essential fatty acids was confirmed by isotopic experiments of R. Schoenheimer and co-workers (e.g. K. Bernhard and R. Schoenheimer, *J. Biol. Chem.* **133,** *707*, *713* (*1940*)) who fed animals a high carbohydrate diet with dilute heavy water. This resulted in formation of deuterium containing palmitic and stearic acids, but no deuterium was present in isolated samples of linoleic or linolenic acids.

K. Bernhard and H. Bodur (*Helvet. chim. acta* **29,** *1782* (*1946*)) determined the effect of feeding fat free rations to pregnant rats on the fatty acid content of the carcass of mother and young. The fat free diet was based on that of Steenbock and co-workers (*loc. cit.*) supplemented with vitamins of the B-complex. This diet was substituted for a stock ration on the thirteenth day of pregnancy and continued for from seven to twelve days after parturition. At this time, mothers and litters were killed, dissolved in a methanol solution of potassium hydroxide, and the fatty acids isolated. The total fatty acid content of the adult animals on the deficient ration was less than half that of the control animals (an average of 3.5 per cent of the body weight, as compared with 7.6 per cent for controls). Young from the control rats contained 7.2 per cent of fatty acids, as compared with an average of 2.0 per cent for the deficient young. The latter animals were also much lighter (average 8.4 g.) than the control animals (average 19 g.). Following separation of the liquid from the solid fatty acids, the linoleic acid content of the former fraction was determined by isolation as the tetrabromide. Control adult animals contained almost ten times as much linoleic acid as the deficient adult animals. No detectable amount of linoleic acid was present in young from the deficient animals; control young contained approximately the same amounts of linoleic acid, expressed on a percentage basis, as their normal mothers.

The study demonstrates that rats deficient in the essential unsaturated fatty acids do not share these with their nursing young. M. Guggenheim and R. Jürgens (*Helvet. physiol. acta* **2,** *417* (*1944*)) had shown that the young obtained on this same deficient diet show severe tail lesions five to eight days after birth without an accompanying acrodynia, and with characteristic alterations in the blood picture. Death occurs, usually toward the end of the third week after birth. On autopsy, such animals show a type of fatty degeneration of the liver with degeneration of the liver cells. This deficiency condition, which was also noted in the present study, can be cured if linoleic acid is administered to the young rats. It is also cured or prevented by administration of linoleic acid in sufficient amounts to the nursing mothers.

THE RELATION OF TYROSINE METABOLISM TO THE SUPPLY OF ASCORBIC ACID

R. R. Sealock and H. E. Silberstein (*J. Biol. Chem.* **135,** *251 (1940)*) observed that feeding tyrosine in amounts of 0.5 g. or more daily to guinea pigs on an ascorbic acid deficient diet results in excretion of homogentisic, p-hydroxyphenylpyruvic, and p-hydroxyphenyllactic acids. Administration of 10 mg. of l-ascorbic acid daily prevented completely the excretion of these metabolites. Evidence of the specificity of the ascorbic acid action was provided by the finding that d-isoascorbic acid was ineffective in replacing an equal weight of l-ascorbic acid, while 200 mg. of d-isoascorbic acid (equivalent in antiscorbutic activity to 10 mg. of l-ascorbic acid) were able to prevent the metabolite excretion.

The same deficit of metabolism which Sealock and Silberstein observed in scorbutic guinea pigs was discovered independently at the same time by S. Z. Levine, H. H. Gordon, and E. Marples (*J. Clin. Invest.* **20,** *209 (1941)*) in premature infants, in whom it could also be abolished by doses of ascorbic acid. It could also be precipitated in full term infants by a single dose of 1.0 g. of phenylalanine or tyrosine, and again abolished by treatment with ascorbic acid.

A later investigation by R. R. Sealock, J. D. Perkinson, and D. H. Basinski (*J. Biol. Chem.* **140,** *153 (1941)*) was made on guinea pigs, testing the urine for tyrosine and certain of its derivatives by the Folin-Ciocalteau reagent, in addition to homogentisic acid and the p-hydroxyphenyl derivatives of lactic and pyruvic acids. Feeding extra l-phenylalanine to ascorbic acid deficient guinea pigs resulted in excretion of tyrosine metabolites as well as the compounds previously observed, the amount of tyrosine derivatives representing a considerable proportion of the supplemental phenylalanine. The greater portion of the tyrosyl value was shown to be p-hydroxyphenylpyruvic acid. Administration of sufficient ascorbic acid rapidly prevented the excretion of all the abnormal metabolites. Essentially similar results were obtained when phenylpyruvic acid was fed, while feeding of p-hydroxyphenylpyruvic acid under the same conditions resulted in metabolite excretion only slightly influenced by administration of ascorbic acid.

In a later report dealing with this problem, D. H. Basinski and R. R. Sealock (*J. Biol. Chem.* **166,** *7 (1946)*) have examined the structural specificity of tyrosine in relation to the metabolic action of ascorbic acid. The immediate objective of the experiments described was the modification, one at a time, of each characteristic feature of the original amino acid structure and the subsequent evaluation of metabolic behavior in the scorbutic guinea pig. Male guinea pigs weighing 300 to 500 g. were used, and ascorbic acid deficiency was induced by feeding a diet of ground Purina rabbit chow (complete ration) which had been rendered almost completely free from ascorbic acid by exposure to the air in thin layers. The compounds under investigation were given as a 10 per cent mixture in the basal diet, which mixture was layered over additional basal diet so that each day's supplement would be entirely consumed. A total of 2.76 mM of each compound was fed, so that it was stoichiometrically equivalent to 0.5 g. of tyrosine. The daily urines were collected in glass jars containing 2 N hydrochloric acid and were analyzed immediately after filtration and dilution. The compounds fed were prepared by standard procedures and purified extensively.

As established in previous publications, l-tyrosine and l-phenylalanine when fed to scorbutic animals gave rise to the appearance of intermediary metabolites in the urine, which promptly disappeared on

administration of ascorbic acid. The first modifications tested were the optical enantiomorphs, and here it was found that when d-tyrosine was fed to the scorbutic animal, approximately 10 per cent appeared in the urine as the alpha-keto acid and somewhat more as the phenolic compound (tyrosyl value). Even more distinctive was the behavior after giving ascorbic acid, even in doses as high as 20 mg. daily. No alteration in these relatively small values occurred; the two facts together indicate that the vitamin exerts its effect only in the metabolism of the natural isomer. Similar results were observed upon feeding d-phenylalanine.

Derivatives of the natural l-amino acids tested were N-acetyl-l-tyrosine, diacetyl-l-tyrosine, N-acetyl-l-phenylalanine, l-p-methoxyphenylalanine, dl-phenylaminobutyric acid, and l-S-benzylcystine. The values for daily excretion of keto-acid and phenolic (tyrosyl) values for every compound showed no correlation with the state of ascorbic acid nutrition, so that it may be concluded that each is metabolized by the guinea pig independently of ascorbic acid. This observation gives added emphasis to the earlier result that the only compounds which show an ascorbic acid-dependent metabolism are l-phenylalanine, l-tyrosine, and phenylpyruvic acid. The dependence upon ascorbic acid is therefore of the highest specificity.

GLUTAMIC ACID ADMINISTRATION AND INTELLECTUAL PERFORMANCE

J. C. Price, H. Waelsch, and T. J. Putnam (*J. Am. Med. Assn.* **122,** *1153 (1943)*) investigated the effects of the administration of dl-glutamic acid hydrochloride to patients with petit mal epilepsy. The glutamic acid was used in conjunction with known anticonvulsant therapy and seizures associated with slow wave activity in the electroencephalogram were decreased in frequency. Grand mal (convulsive) seizures were unaffected by the drug. Of interest for the present review were striking changes in the mental and physical alertness of the subjects which were regularly noted and could not be correlated with the incidence of seizures. Improvements in personality were again found in a subsequent study by H. Waelsch and J. C. Price (*Arch. Neurol. Psychiat.* **51,** *393 (1944)*) in which there was evidence that such a therapeutic effect was associated with natural l (+) glutamic acid administration.

The suggestion that the administration of natural l (+) glutamic acid, present in body protein and in food, might facilitate mental functioning led to several animal studies. F. T. Zimmerman and S. Ross (*Arch. Neurol. Psychiat.* **51,** *446 (1944)*) administered glutamic acid supplements to rats and compared their performance on a maze with that of controls fed only a basic diet. The level of glutamic acid supplement was approximately equal to that in the food (Chick Growena), the experimental animals thus receiving twice that of the controls. Statistically significant differences were found between the experimental and control group. Trials, time, and error scores were compared. Rats fed l (+) glutamic acid learned the maze in fewer trials, with fewer errors, and in a shorter time than control rats fed only the basal diet. Similar growth curves in the two groups indicated that such differences could not have been due to inanition.

A problem box was used in the Columbia Laboratories by K. E. Albert and C. J. Warden (*Science* **100,** *476 (1944)*) to test the effect of the amino acid on the learning of 8 rats. Such a test of performance presented a series of increasingly difficult problems, and was considered a test of intelligence level. Supplemented rats advanced further on this series of problems

than the controls. These animals, similar to those of the previous experiment, were healthy animals receiving a basic diet thought to be adequate for normal development.

M. H. Marx (*Am. Psychologist* **1,** *273 (1946)*) published a brief report of experiments in which negative results were obtained. Groups of 45 rats were used and 200 mg. of 1 (+) glutamic acid were given the experimental animals by stomach tube. Learning tests in a water-maze failed to show any significant differences of performance.

Suggested by the animal studies and the observation of behavior changes in epileptic patients, two groups of investigators reported the effect of 1 (+) glutamic acid upon the intellectual performance of mentally retarded patients. K. E. Albert, P. Hoch, and H. Waelsch (*J. Nerv. Ment. Dis.* **104,** *263 (1946)*) administered glutamic acid to 8 individuals suffering from mental deficiency. The intelligence quotients (IQ) as measured by the Stanford-Binet test ranged from 22 to 73. The subjects ranged in chronologic age from 6 to 26 years while their mental ages ranged from 2 to 8 years. A well standardized battery of psychologic tests—intelligence, memory, manipulative skill, and personality—were administered. While it was not possible to give the same tests to all subjects (owing to variations in mental age) this was not thought to be of great importance as they were compared with themselves. After the performance levels of the subjects were determined they were given either placebos or glutamic acid averaging 18 tablets daily of 0.5 g. each. Some were first placed on placebo medication and later changed to glutamic acid while the procedure was reversed for the others.

As measured by the tests 7 out of the 8 patients studied showed raised levels of mental functioning after the oral administration of glutamic acid in amounts from 8 to 10 g. per day. The effect was dependent upon the continuous administration of the amino acid and patients reverted to previous levels of performance when placebos were given. The tendency to relapse was not immediately discernable after terminating the medication.

Detailed case histories of 4 patients were given. An example of mental changes associated with glutamic acid administration was that of a 14 year old, undersized, imbecilic boy with an IQ of 42. In the seven and a half years before glutamic acid treatment the patient had gained an average of 4½ months of mental age per year. In two months on glutamic acid medication the patient gained 1 year and 5 months in mental age or two thirds of his total gain during the previous seven and one-half years. The patient reverted to his previous mental level when placebos were substituted for the glutamic acid. The other patients showed similar unmistakable and measurable increases in mental performance. The authors felt that such changes could not be ascribed to maturation or other environmental influences. Universal drops in performance to premedication levels when placebos were given indicated that the gains were valid and did not result from increased familiarity with the tests. New but comparable tests were usually used for retests.

A second study of glutamic acid and mental functioning has been made by F. T. Zimmerman, B. B. Burgemeister, and T. J. Putnam (*Arch. Neurol. Psychiat.* **56,** *489 (1946)*). Seven out of 9 subjects in this study were patients with convulsive disorders, 2 were mentally retarded without convulsions. A battery of well standardized mental performance tests was given at the beginning and at the end of a six month period during which the patients received 1 (+) glutamic acid. The doses of glutamic acid averaged 12 g. daily although individual patients differed in their tolerance. The amino acid was usually given in increasing

amounts until increased motor activity or gastric distress was apparent.

Sharp positive increments in mental age (IQ average increases of eight points) were apparent with the performance of low grade subjects, increasing at a rate faster than would be expected of normal children. The seizures of the control subjects were reduced by anticonvulsant drugs other than l (+) glutamic acid. Part of the improvement in the retest scores of both experimental and control groups was attributed to reduction of seizures.

Both of the foregoing studies obtained evidence of improvement in personality on certain tests as well as in verbal and performance test scores. Dull and inattentive patients after medication became more alert, attentive, and persistent in trying to solve the test problems confronting them. It seemed that the improved performances were due to a better utilization of existing abilities rather than an acquisition of new intellectual tools (increases in intelligence). Motivation and emotional factors play an important role in the ability to perform intellectually.

Albert, Hoch, and Waelsch (*loc cit.*) indicated that glutamic acid may also shorten the periods of memory loss and confusion in psychotic patients after undergoing electro-shock treatments. These investigators felt that glutamic acid probably does not increase intellectual capacity but may enable the individual to regain intellectual functions inhibited by other factors.

It is not known at present whether a glutamic acid deficiency was being overcome by the administration of the amino acid. This possibility would be of great interest from a nutritional point of view since glutamic acid is present in food and also synthesized in the body. In the studies under consideration detailed information is not included on the analyses of the basal diets the patients received. Knowledge of and control over the diet of institutionalized patients will be necessary before the effects of glutamic acid supplements can be clearly evaluated.

Studies by H. Weil-Malherbe (*Biochem. J.* **30,** *665 (1946)*) indicate that 1(+) glutamic acid is the only amino acid metabolized by brain slices. Furthermore D. Nachmansohn, H. M. John, and H. Waelsch (*J. Biol. Chem.* **150,** *485 (1943)*) suggest that the metabolism of acetylcholine is intrinsically related to electrical changes during neuron activity. The energy for such action potentials seemed to derive from phosphate compounds yielding energy for the formation of acetylcholine. Enzyme systems associated with the synthesis of acetylcholine in brain extracts, when inactivated by dialysis, were partially reactivated by the addition of glutamic acid. No precise mechanism is yet known for the interaction of glutamic acid and acetylcholine. The foregoing evidence, however, suggests that the physiologic basis of the observed effects of glutamic acid may be related to the formation of acetylcholine.

In the past, mental defects have been considered unalterable. The foregoing studies open an entirely new avenue of approach and suggest that improvement of brain function may be approached by biochemical means.

NUTRITIONAL ASPECTS OF CERTAIN ENDOCRINE DISTURBANCES

Evidence that an important function of the liver is concerned with the inactivation of certain hormones is accumulating. This is of interest to those concerned with nutrition since nutritional deficiency may lead to impairment of liver function.

To study the metabolism of the sex-endocrine steroids, G. R. Biskind (*Proc.*

Soc. Exp. Biol. Med. **43,** *259* (*1940*)) devised a technic which can be used in the intact animal. By placing a pellet of estrone, estradiol, or estradiol benzoate in the spleen of a castrate rat, the absorbed steroid must pass through the liver before reaching the systemic circulation. In normal animals when this was done no estrogenic effect was demonstrable, nor was there an androgenic effect when pellets of testosterone, testosterone proprionate, or methyl testosterone were placed in the spleen. This suggested that the livers of rats inactivated both estrogens and androgens. Transplantation of the spleen containing the pellet of steroid and ligation of the pedicle after establishment of a collateral circulation produced the specific estrogenic or androgenic effect in accord with the nature of the implanted pellet (G. R. Biskind, *Ibid.* **46,** *452* (*1941*)).

M. S. Biskind and G. R. Biskind (*Endocrinology* **31,** *109* (*1942*); **32,** *97* (*1943*)) then studied the effects of pellets of estrone implanted in the spleens of rats whose diet had been depleted in B-complex vitamins. Castrated female rats with such pellets in their spleens remained anestrus when receiving a normal diet, but went into continuous estrus when the diet was depleted. Addition of brewers' yeast or a mixture of crystalline vitamins to the diet restored the anestrus state. Subsequent depletion of the vitamins again led to continuous estrus. It is of interest that this impairment of the inactivating mechanism in the liver bore no necessary relation to detectable morphologic changes in this organ.

In a general discussion of these observations and their implications, G. R. Biskind and M. S. Biskind (*Am. J. Clin. Path.* **16,** *737* (*1946*)) refer to their numerous papers on this subject and point out that they have observed in acute experiments in the rat that thiamine and riboflavin alone among the B-vitamins are adequate to permit hepatic destruction of estrogen and that the presence of methionine appears essential to this function. Furthermore, in contrast to the estrogens, deficiency of the B-vitamins has not been associated with any impairment of the ability of the liver to destroy the masculinizing hormone, androgen. Consequently it could be postulated that nutritional deficiency and hepatic functional impairment may lead to an alteration of the estrogen-androgen equilibrium which exists normally.

That the liver in man is concerned with inactivation of hormones is suggested by the observation that in male patients with cirrhosis of the liver, urinary estrogen is increased and appears in the free form while urinary androgen is reduced and is present only in combined form (S. J. Glass, H. A. Edmondson, and S. N. Soll, *J. Clin. Endocrinology* **4,** *54* (*1944*)). It is not unusual to see in such patients testicular atrophy or large breasts or both. The Biskinds point out that, since metabolism of the androgens is not significantly affected by the function of the liver, a serious alteration in the estrogen-androgen equilibrium in the body can occur when liver function is impaired, with retention of body estrogen. Since it has been postulated (R. T. Frank, M. A. Goldberger, and F. Spielman, *J. Am. Med. Assn.* **103,** *393* (*1934*)) that excessive menstrual bleeding, premenstrual tension, cyclic painful enlargement of the breasts, chronic cystic mastitis, uterine myomas and other benign overgrowths, or even malignant neoplasms of such tissues as the uterus and breasts may be the result of excess estrogen production, unpleasant and even serious consequences could be the result of such retention of body estrogen.

These experiments and the speculation outlined form the rationale for a kind of therapy which the Biskinds have employed in more than 700 patients. These patients presented such evidence of nutritional deficiency as glossitis and cheilosis and complained of symptoms ranging from

simple premenstrual tension to severe irregular and excessive bleeding. The response to "intensive, persistent, and complete" nutritional therapy was invariably excellent in all the syndromes related to excess estrogen. In male subjects, testicular atrophy, diminution in libido and potency infertility, and enlargement of the breast were likewise found to be responsive to such therapy.

A similar concept has been applied by the Biskinds to the management of diabetes on the ground that the liver plays a basic role in the maintenance of normal carbohydrate metabolism. Partial or complete restoration of carbohydrate balance occurred in 94 patients receiving intensive and persistent nutritional therapy, in 25 patients striking reductions in blood sugar levels occurred on nutritional therapy alone, the insulin requirement was reduced in 14, eliminated in 16, and in 37 in whom the insulin requirement was unchanged the improvement in general health and well-being was striking.

The concept expressed by the Biskinds is an interesting one and possesses some experimental basis. As pointed out above, there is also some evidence in man as well as in the rat that the liver is concerned with the inactivation of estrogens. For this view to be acceptable as a basis for therapy, however, it is necessary to have carefully controlled studies supported by objective methods of measurement of any effects which may be produced. Unfortunately it is extremely difficult to step with assurance from the experimental animal observed under easily controlled conditions, to the patient in whom so many factors, physical as well as psychologic, play a part.

DENTAL CARIES ACTIVITY IN MALNOURISHED PATIENTS

Various relationships between diet and dental caries activity have been discussed (*Nutrition Reviews* **5,** *68 (1947)*). A. W. Mann, S. Dreizen, T. D. Spies, and F. M. Hunt (*J. Am. Dent. Assn.* **34,** *244 (1947)*) compared the dental caries activity in 124 patients diagnosed clinically as malnourished and in 99 well nourished individuals. Clinical evidence of nutritional deficiency disease was determined from medical examinations and physical findings of the physicians and dentists of the nutrition clinic. The lesions and signs regarded as indicative of nutritional deficiencies were stated to be "of vitamin A—Bitot's spots, nyctalopia or night blindness, xerophthalmia, and hyperkeratosis of the hair follicles; of vitamin B_1 (thiamine)—nutritional peripheral neuritis (calf tenderness) associated with or without edema; of vitamin B_2 (riboflavin)—angular cheilosis, so-called magenta tongue and vascular injections of the conjunctiva and the sclera of the eye accompanied by photophobia, which responded to riboflavin therapy; of niacin—pellagrous glossitis, pellagrous dermatitis and gastrointestinal disturbances. Subclinical pellagra was suggested by the initial nervous syndrome in which the patient exhibited fatigue, insomnia, anorexia, vertigo, burning sensations in various parts of the body, numbness, palpitation, nervousness, a feeling of unrest and anxiety, headache, forgetfulness, apprehension and distractability, and visual and auditory pseudohallucinations which were specifically relieved by niacin; of ascorbic acid-scorbutic gingivitis characterized by reddened gingivae which bled easily when touched, subperiosteal petechial hemorrhages, ecchymosis, and a lack of ascorbic acid in the plasma; of nutritional macrocytic anemia with a high color index in a patient who had free hydrochloric acid, pepsinogen, and rennin in the gastric juice. There were no signs of rickets in any of the patients

studied." Clinical findings on the 124 malnourished patients indicated that 34 had evidence of pellagra, 84 of riboflavin deficiency, 29 with the initial nervous syndrome, 15 with nutritional macrocytic anemia, 27 with clinical evidence of thiamine deficiency, 11 with vitamin A deficiency, and 17 with clinical scurvy. Sixty-two of these patients had clinical evidence of multiple deficiencies. No clinical evidence of nutritional deficiency diseases was found among the 99 control subjects.

Dental examinations were made with the aid of mouth mirror, exploring tine, and bitewing or full mouth roentgenograms. The malnourished patients had an average of 2.07 tooth surfaces decayed, 1.60 teeth missing, and 0.96 tooth surfaces filled, while the control individuals had an average of 3.21 tooth surfaces decayed, 2.23 teeth missing, and 9.75 tooth surfaces restored. The average of decayed, missing, and filled tooth surfaces for the malnourished group was 4.54 and for the control group 14.94. Thus the group of subjects diagnosed clinically as deficient in vitamin A, thiamine, riboflavin, niacin, or ascorbic acid, or deficient in two or more of these nutrients simultaneously, had a life dental caries experience of only about 30 per cent of the control subjects. Three laboratory tests which have been proposed by various investigators as indicators of dental caries activity in subjects at the time of the test were employed as comparing mechanisms for these two groups of subjects. The free calcium determination in the saliva before and after incubation (L. S. Fosdick, H. L. Hansen, and C. Epple, *J. Am. Dent. Assn.* **24,** *1275 (1937)*), the Lactobacillus acidophilus counts on tomato juice agar plates (F. P. Hadley, *J. Dental Research* **13,** *415 (1933)*), and the buffer capacity test (S. Dreizen, A. W. Mann, J. K. Cline, and T. D. Spies, *Ibid.* **25,** *213 (1946)*) yielded results which in the interpretation of their proponents would indicate a lower dental caries activity in the group of malnourished subjects than in the group of well nourished individuals.

These data of Mann *et al.* substantiate and extend the results of A. H. Kniesner, A. W. Mann, and T. D. Spies (*J. Dental Research* **21,** *259 (1942)*) who studied the dental caries experience of 41 patients hospitalized for treatment of vitamin B-complex deficiencies and concluded that their dental caries experience was as low or lower than in normal individuals of the same age groups.

No statement was made in these studies of how long the duration of these deficiencies was, and indeed the probability is that there was no way in which this could be even roughly estimated as the malnourished subjects were probably placed on curative dietary regimens shortly after clinical diagnosis. Since measurements of decayed, missing, and filled tooth surfaces represent the accumulated dental caries experience of the individual, the lower dental caries experience of the malnourished individuals would imply that these individuals had had a lower dental caries susceptibility throughout life. Whether the low dental caries susceptibility had been associated with prolonged clinical or subclinical nutritional deficiencies cannot be determined from present knowledge. It is evident that there is no rampant dental caries activity during periods of single or multiple deficiencies of ascorbic acid and vitamin A, and the members of the vitamin B-complex studied. Thus any tendency of large numbers of the human race to be deficient in the factors studied in these investigations would not seem to be related to the present high incidence of dental caries.

THE PHENYLALANINE REQUIREMENT OF THE RAT

Just a decade ago, when in the course of his classic researches on feeding of rats with mixtures of amino acids in place of proteins he first succeeded in obtaining growth on a mixture of highly purified amino acids, Professor W. C. Rose published a preliminary account of his results (*Science* **86,** *298* (*1937*)). In the following year he published a review of the whole subject (*Physiol. Rev.* **18,** *109* (*1938*)) although the detailed evidence on which many of his statements were based had not all appeared. The evidence relating to phenylalanine has now appeared (W. C. Rose and M. Womack, *J. Biol. Chem.* **166,** *103* (*1946*)), and Dr. Rose mentions that in addition to other delays, wartime duties have caused this detailed evidence to be withheld until the present.

In reviewing earlier work on the availability of the unnatural as well as the natural stereoisomers to the rat, the authors cite the work of V. du Vigneaud, R. R. Sealock, and C. Van Etten (*J. Biol. Chem.* **98,** *565* (*1932*)) and C. P. Berg (*Ibid.* **104,** *373* (*1934*)) as showing that the optical isomers of tryptophane possess equal nutritive value for the rat. G. J. Cox and C. P. Berg (*Ibid.* **107,** *497* (*1934*)) showed that for histidine the d(+) form is only slightly less effective than the "natural" l(−) isomer, while R. W. Jackson and R. J. Block (*Ibid.* **122,** *425* (*1937–38*)) found that both l(−) and d(+) methionine can successfully supplement diets low in methionine and cystine.

In the first experiments reported in the paper of Rose and Womack (*loc. cit.*) the nitrogen of the basal diet was supplied as a mixture of amino acids devoid of phenylalanine, tyrosine, and threonine. Threonine was added separately to the diet as the natural isomer, and when a racemic (synthetic) amino acid was used in the mixture the quantity was doubled to allow for possible failure of utilization of the unnatural enantiomorph. The basal diet contained 18 per cent of natural amino acids including threonine, and when one of the isomers of phenylalanine was added, the total quantity of natural amino acids was maintained at 18 per cent by an appropriate reduction in the quantity of other amino acids used. In addition to all the essential amino acids except phenylalanine, the mixture of amino acids supplied by the basal diet included glycine, alanine, serine, cystine, proline, hydroxyproline, and aspartic and glutamic acids. The basal diets provided in addition to the amino acids dextrin, sucrose, salt mixture, agar, lard, and cod liver oil. The vitamin B factors were provided in the form of 2 pills daily, each containing 75 mg. of Vitab products, presumably a mixture from natural sources.

The purity of the phenylalanine isomers used was established by correct analytical data and by the optical rotation. The effects of the two isomers as supplements to the basal diet were tested on 10 rats of 2 litters. No information on the percentage of dietary phenylalanine necessary to induce satisfactory growth in the rat was available when this experiment was performed, so that only 0.5 per cent of phenylalanine was included in the diet for the first eight days of the test. This quantity proved to be insufficient, and the rats all lost weight, although they lost less than the controls deprived of phenylalanine.

After the first eight days the level of phenylalanine added to the diet was raised to 1.0 per cent, and growth began at once in animals receiving either isomer. The rate of gain was slightly greater with the natural isomer, but the significance of the difference was doubtful.

In a second series of experiments a new amino acid mixture was employed. This mixture supplied exactly the same compounds, but generally in smaller quantities, so that 16.7 per cent of the mixture (of which 10.5 per cent would be in the physiologically

active form) was employed in the basal diet. In this series it was possible to supply the vitamin B factors in more nearly synthetic form, either as 150 mg. of milk concentrate and 100 mg. of tikitiki extract, or as 10 micrograms of thiamine hydrochloride, 20 micrograms of riboflavin, 10 micrograms of niacin, 10 micrograms of pyridoxine hydrochloride, 50 micrograms of calcium pantothenate, 5 mg. of choline chloride, 1.5 mg. of para-aminobenzoic acid, 25 mg. of wheat germ oil, and 12.5 mg. of Wilsons' liver powder 1:20 daily. No difference was observed between the growth of rats on these two vitamin supplements. In this series addition of phenylalanine was at the expense of dextrin rather than the other amino acids.

The rats were subdivided into groups which received different amounts of phenylalanine from 0.5 to 1.2 g. daily, in an effort to determine the minimum amount which produced maximum growth under the conditions used. Seventy-five of the rats were given dl-phenylalanine and 37 received l-phenylalanine. Maximum growth could be attained when either dl- or l-phenylalanine was fed, and there was some indication that on higher levels of dl-phenylalanine, growth was somewhat less. The latter point remains undecided until more rats can be tested on the highest levels used.

In the preliminary paper referred to above (*loc. cit.*) a list of the essential amino acids had been given, stating the minimum amount of phenylalanine required for growth of the rat to be 0.7 per cent of the diet. It was of course emphasized that the figures then presented were strictly tentative, and it now appears that this figure for phenylalanine should be raised to 0.9 per cent, a figure which is no longer to be regarded as tentative, so long as the diet provides an abundance of nonessential amino acids but no tyrosine.

The last qualification must be made in view of a further study by M. Womack and W. C. Rose (*J. Biol. Chem.* **166,** *429 (1946)*) in which the minimal level of phenylalanine was determined when some tyrosine was also present in the diet. Earlier claims for the interconversion of phenylalanine and tyrosine in the body had been shown to be inadmissible, and the inability of tyrosine to replace all the phenylalanine of the diet had been clearly established by M. Womack and W. C. Rose (*Ibid.* **107,** *449 (1934)*). As however, A. R. Moss and R. Schoenheimer (*Ibid.* **135,** *415 (1940)*) obtained evidence that the reverse change of phenylalanine can occur in the body it left open the possibility that the inclusion of tyrosine in the food could lower the phenylalanine requirement by sparing the conversion of the latter into the former. The experiments now reported (*loc. cit.*) confirm this hypothesis, and indicate that approximately half of the phenylalanine can be replaced by its p-hydroxy derivative.

Growth experiments similar to those described above were performed but with three different types of vitamin B-complex supplements. When the diet contained 0.5 per cent of phenylalanine (a suboptimal level), further addition of 0.8 per cent tyrosine exerted a marked effect on the growth rate, whereas in the absence of phenylalanine, tyrosine had no effect.

INCREASING THE RIBOFLAVIN CONTENT OF HENS' EGGS

S. Lepkovsky, L. W. Taylor, T. H. Jukes, and H. J. Almquist (*Hilgardia* **11,** *559 (1938)*) and other investigators demonstrated a direct relationship between the riboflavin content of the ration fed to laying hens and the riboflavin concentration of the eggs produced. L. C. Norris and J. C. Bauernfeind (*Food Research* **5,** *521 (1940)*) observed that the riboflavin concentration of hens' eggs reached a maximum at an

intake of 800 to 1000 micrograms of riboflavin per 100 g. of ration (3600 to 4500 micrograms of riboflavin per pound of ration). Until the investigations of S. H. Jackson and co-workers (*J. Nutrition* **32,** *567 (1946)*), no data had been presented to indicate the balance between riboflavin intake and the output in the eggs nor the effect of different levels of egg production on the riboflavin output. In the first experiment the riboflavin recovery in eggs laid by groups of hens receiving, respectively, 985, 1310, 1600, and 2230 micrograms of riboflavin per pound of ration was studied over a fourteen week period after a four week stabilization period. The average riboflavin content of the eggs laid by the four groups of hens was 105, 148, 178, and 178 micrograms per egg, respectively. Egg production was higher in the second and third groups. The average percentage of riboflavin recovered in the eggs from the four groups of hens was 18.9, 24.2, 21.9, and 14.8, respectively. Thus the riboflavin concentration in the ration which gave the highest concentration of riboflavin in the eggs was about 1600 micrograms per pound of food, while the riboflavin concentration in the ration which was found to be most efficient in terms of riboflavin output was 1300 micrograms per pound. The groups of hens described above were kept in pens where no limitation could be put on their physical activity. In a comparative experiment done simultaneously, hens kept in laying batteries were found to be considerably more efficient in converting riboflavin from the ration to their eggs than those hens kept in pens. The average efficiency was about 7 to 8 per cent higher for the hens housed in laying batteries.

In another experiment these workers followed the riboflavin output and the egg production of a larger number of hens through an eight month period from November through June. In this case three rations were employed which contained 1185, 1870, and 2560 micrograms of riboflavin per pound, respectively. A relatively large increase in total riboflavin output was observed from February through March. This was mainly due to an increase in total egg production but was also partially contributed to by an increased riboflavin concentration in the eggs. Throughout the experiment the hens which consumed the ration with the medium level of riboflavin produced eggs of higher riboflavin content than those hens which received the lowest level of riboflavin. During the period of greatest egg production, the eggs produced by the hens consuming the high riboflavin ration had the highest riboflavin content; during the periods of lower egg production, this ration was not found to be superior to the ration with the medium level of riboflavin. Thus from these data, a riboflavin concentration of 1600 micrograms per pound of feed would seem to be ordinarily adequate for the maintenance of an efficient, high level of riboflavin in the eggs. However, during maximum egg production, the upper effective level of riboflavin was greater than 1870 micrograms per pound of ration and possibly as high as 2560 micrograms. The latter level approaches the amounts which were suggested by Norris and Bauernfeind (*loc. cit.*) as the maximum intake which would influence the riboflavin content of the eggs.

Jackson and co-workers also studied the variation in riboflavin content of different hens and different breeds of hens. The riboflavin concentration was affected somewhat by individual and breed characteristics but the differences were not regarded as important in practical egg production. The sample of eggs taken for the study of breed variation consisted of thirty eggs from each of twelve breeds of hens on the practical ration containing 1380 micrograms of riboflavin per pound. The extremes were 139 micrograms per egg for a breed known as New Ontario and 198 micrograms per egg for White Leghorns. This represents

an increase of almost 50 per cent, and if the samples were truly representative of the breeds tested, the investigators' conclusion that these differences were not of practical significance would seem to be somewhat conservative. The value for the Barred Plymouth Rocks used in the above experiments lay almost at the mean of the twelve breeds studied.

Actually the increase in riboflavin content of eggs to be achieved either through the fortification of the laying hens' diet with riboflavin concentrates or through careful selection of the breed of hen is not great in terms of the daily human riboflavin requirement. In the communities where there is the greatest likelihood of borderline riboflavin deficiencies, the dietary probably is almost completely void of eggs for economic reasons.

SARCOSINE AS A DONOR OF LABILE METHYL GROUPS

V. du Vigneaud, S. Simmonds, and M. Cohn (*J. Biol. Chem.* **166,** *47 (1946)*) made a study of transmethylation with isotopic sarcosine (methylglycine) as a methyl donor. Isotopic sarcosine was fed to 4 rats for seven days as a supplement to an otherwise methyl-free diet containing homocystine, following which the rats were killed for the isolation of choline, creatine, glycine, and glutamic acid from the tissues. To determine the relative efficiency of sarcosine as a methyl donor, a control experiment was run in which isotopic choline was fed instead of sarcosine. The isotopic sarcosine used contained N^{15} and also deuterium in the methyl group; the choline used contained deuterium in the methyl groups but no isotopic nitrogen.

Isotope analyses showed that sarcosine had caused a slight transfer of methyl groups to choline and creatine, although it was not an effective methyl donor for the synthesis of these compounds. During the course of the seven days of isotopic sarcosine feeding, about 1 to 2 per cent of the methyl groups of choline and about 0.5 per cent of the methyl group of creatine were derived from the sarcosine. As a methyl donor the sarcosine compared unfavorably with choline, and the results with sarcosine feeding suggested a sex difference which required further investigation. When the amounts of isotopic derivatives were expressed in terms of the weights of the individual animals (per mM of test compound fed per 100 g. of body weight), the creatine N^{15} values resulting from the feeding of isotopic sarcosine appeared to exhibit a random fluctuation of about ± 10 per cent in different rats. Compared with this, the 2 female rats contained creatine in which about 70 per cent more methyl groups had been derived from sarcosine than in the samples isolated from the male rats.

No such difference between the results of feeding isotopic choline to the male and female rat was observed. From a comparison of the percentage of methyl groups and nitrogen in the isolated choline derived from the fed sarcosine, it was possible to calculate that the sarcosine molecule is not converted to choline as a whole; in the same way it was also shown that the sarcosine molecule is not converted intact to creatine, but is first demethylated to yield glycine. This is in agreement with the results of K. Bloch and R. Schoenheimer (*J. Biol. Chem.* **135,** *99 (1940)*; **138,** *167 (1941)*) and of H. Borsook and J. W. Dubnoff (*Ibid.* **138,** *389 (1941)*).

Comparing the results reported above for sarcosine with those previously reported by V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn (*J. Biol. Chem.* **165,** *639 (1946)*) for dimethylglycine and betaine, some idea of the relative efficacy of the three compounds as methyl donors can be obtained. Although the experimental con-

ditions were not identical, the activity of choline was measured as a reference in each case. Such a comparison shows that betaine is the most active of the three glycine derivatives, whereas dimethylglycine and sarcosine are relatively inactive in supplying methyl groups. This is in line with the observation that betaine is the only one of the three compounds which can replace choline in growth tests with the rat (V. du Vigneaud, J. P. Chandler, A. W. Moyer, and D. M. Keppel, *Ibid.* **131,** *57* (*1939*); and A. W. Moyer and V. du Vigneaud, *Ibid.* **143,** *373* (*1942*)).

CANINE HYSTERIA AND AGENIZED FLOUR

Sir Edward Mellanby (*Brit. Med. J.* **2,** *885* (*1946*)) has published a report of an investigation which promises to clarify the origin of "canine hysteria." Dogs subject to attacks of hysteria are described by Mellanby as being less interested in their surroundings, as walking or running more slowly, with a tendency to raise their forelegs abnormally high while walking, and to "bounce along" when running. The animals have dry mouths, avoid sun, and are less friendly and more readily frightened than normal dogs. The attacks appear to be precipitated by sudden stimuli such as loud noises or by change of environment or nervous strain. They recur at intervals of several days, or a number of attacks may develop within a single day. The attacks of slight hysteria are manifest by a frightened appearance and the animal stands with the legs somewhat apart and the ears back. Backward jerking of the head may occur. The dog may dash across the cage into the wall and then suddenly sit down and become quiet. In the most severe cases the jerking of the head is followed by a similar movement throughout the body, the animal then begins an aimless running round and round its cage, sometimes barking furiously, and may dash into the wall or overturn vessels in the cage seemingly without seeing the objects. Such an hysterical fit may continue until collapse results.

The earlier studies indicating a probable relationship between wheat gluten and this disease have been reviewed (*Nutrition Reviews* **3,** *176* (*1945*)). Mellanby (*loc. cit.*) states that in 1931 the concept had developed that outbreaks of hysteria were due to the bread portion of the diet. The basis of this conclusion was that dogs had never developed hysteria when oatmeal formed the major portion of the diet, and, further, that outbreaks of hysteria could be terminated by replacing half or more of the bread by oatmeal. During the war period the Cereal Division of the Ministry of Food arranged to have supplies of flour for testing obtained from a single mill. The cooperating miller examined some of the flours, the consumption of which had been associated with canine hysteria, and noted that they had been "heavily bleached and probably improved." Since a high percentage (an estimated 90 per cent) of the flour used for bread making in Britain was stated to be bleached by the agene process in which nitrogen trichloride gas is used, and since this gas is known to affect the gluten of the flour, it was suspected that this process might bear a relationship to canine hysteria.

To test whether such a relationship existed, young dogs of the same litter were fed diets containing agene-treated and untreated samples of flour from the same lot of grain. The diet employed was one known to be adequate and compatible with normal growth and health provided untreated flour or oatmeal were used as the cereal. The diet consisted of skimmed milk powder, 20 g.; cereal, 50 to 340 g.; lean meat, 15 g.; peanut oil, 10 ml.; bakers' yeast, 5 per cent of the cereal; ascorbic acid, 5 mg.; sodium chloride, 1 to 2 g.; vitamin A, 2000 or 3000

I.U.; and vitamin D_2, 200 I.U. The dietary cereal was steam cooked for ninety minutes. When agenized flour was the source of cereal in the diet, growing dogs with large appetites developed canine hysteria after a period of two weeks or less of feeding. Control animals receiving unbleached flour did not develop hysteria. The fits ceased suddenly upon changing the flour from the bleached to the unbleached variety in the diet of badly affected animals.

The data indicating this relationship are convincing. Thus, during a period of twenty-four weeks one dog receiving untreated flour had no hysteria or fits. During the same period, the litter mate control receiving the treated flour had thirty attacks. The diets were then reversed and during the second period, which lasted for twelve weeks, the dog which had initially received the untreated flour and had remained free of attacks developed twenty-four attacks, and the animal previously manifesting attacks had none during the period of feeding on the untreated cereal.

In one series of experiments the vitamin A was omitted from the daily diet. When agenized wheat flour was used in this vitamin A deficient diet the animals likewise developed canine hysteria. Thus, it was demonstrated that canine hysteria could be superimposed on vitamin A deficiency. Mellanby suggests that it is possible that canine hysteria has sometimes been mistaken for and perhaps even superimposed on such conditions as vitamin A or vitamin B-complex deficiency.

It is of considerable importance that the substitution of untreated flour for the treated flour reversed the animal's condition and permitted complete recovery from the hysteria and fits, but that the "animal itself may not be normal." The longer the exposure to the diet, the more slowly the animal returned to normal upon the substitution of untreated flour.

Canine hysteria has been shown to be of dietary origin, and, in particular, to be associated with a high content of wheat gluten in the diet. Mellanby states that it has also been held that the agene process affects the gluten of flour, probably by the entrance of chlorine into the tyrosine or tryptophane groupings. The experiments here described seem to demonstrate that agenized flour exhibits a toxic effect on the dog which results in canine hysteria. Thus, a question raised in the previous review of this subject appears to have been answered. These observations in dogs do not necessarily indicate a toxicity for man as indicated by the fact that the agenized flour was not toxic for rats. Furthermore, J. R. Wagner and C. A. Elvehjem (*J. Nutrition* **28,** *431 (1944)*) could not produce symptoms in the rat, mouse, chick, guinea pig, or monkey by feeding diets high in wheat gluten.

THE ASCORBIC ACID OF THE BODY

With the development of ultra-micro-methods of analysis for ascorbic acid (*Nutrition Reviews* **3,** *336 (1945)*) new and extended possibilities have been opened for the collection of data recording the ascorbic acid content of whole blood, plasma, white cells, or red cells. Since measurements of plasma ascorbic acid levels were first possible, attempts have been made to apply such measurements, either of fasting plasma levels or of levels following specified "load treatments," to the diagnosis of scurvy or to the evaluation of ascorbic acid nutrition. It was early learned that the fasting plasma ascorbic acid level was not a good guide to the condition of a subject with regard to ascorbic acid, although some workers at various times set up standards and diagnosed

subclinical scurvy in subjects whose fasting level was below 0.6 mg. ascorbic acid per cent, or scurvy in those whose levels were below 0.3 mg. per cent, even in the absence of clinical symptoms or signs.

Of the "load" or "saturation" tests which were devised a number gave quantitative information regarding the "saturation" of the subject with ascorbic acid, and several were claimed to be of value in diagnosing scurvy. Perhaps the most satisfactory was proposed by L. Kajdi, J. Light, and C. Kajdi (*J. Pediatrics* **15,** *197 (1939)*) who observed a correlation between their load test and the diagnoses made by clinicians, and concluded that they could, with considerable confidence, make a diagnosis from the results of their test. Essentially the test consisted in the measurement of the plasma ascorbic acid of infants four hours after the intramuscular injection of 200 mg. of ascorbic acid.

The use of the ultra-micromethods introduced by O. A. Bessey and O. H. Lowry (*Am. J. Pub. Health* **35,** *941 (1945)*; *Nutrition Reviews* **3,** *336 (1945)*) has focused attention again upon the distribution of the ascorbic acid in the blood. The white blood cells were shown to have a higher concentration of ascorbic acid than the plasma or any other cellular elements of blood by D. J. Stephens and E. E. Hawley (*J. Biol. Chem.* **115,** *653 (1936)*). The possible nutritional significance of the ascorbic acid of the white blood cells was realized sometime ago by A. M. Butler and M. Cushman (*J. Clin. Invest.* **19,** *459 (1940)*), who observed that subjects in a poor state of ascorbic acid nutrition with little or no ascorbic acid in their plasma could still have considerable amounts in the white blood cells. J. H. Crandon, C. C. Lund, and D. B. Dill (*New Engl. J. Med.* **223,** *353 (1940)*) had shown that in a single subject maintained on a scorbutic diet the serum level of ascorbic acid fell almost to zero within three or four weeks, whereas the white cells contained about 30 mg. per cent at the beginning of the experiment, and this level fell slowly until it reached zero over four months after the plasma level had been zero, and only a week or two before clinical scurvy appeared.

An extensive study has now been made of the serum and white cell ascorbic acid levels of a group of 100 members of the Royal Canadian Air Force who were maintained on controlled diets of known ascorbic acid content (O. H. Lowry, O. A. Bessey, M. J. Brock, and J. A. Lopez, *J. Biol. Chem.* **166,** *111 (1946)*). The authors suggest that the data of Crandon, Lund, and Dill (*loc. cit.*) "have created the impression that the white cells, and hence presumably the other tissues, contain undiminished amounts of the vitamin as long as ascorbic acid is demonstrable in the serum," and they point out that results in support of this hypothesis have been reported by M. Pijoan and E. L. Lozner (*Bull. Johns Hopkins Hosp.* **75,** *303 (1944)*). On the other hand this hypothesis seems to be contradicted by the common observation that considerable amounts of ascorbic acid may be retained in a load test by subjects with low but definite serum ascorbic acid concentrations.

The details of the R. C. A. F. experiment whose personnel were observed by the authors have been given by W. J. Linghorne *et al.* (*Canad. Med. Assn. J.* **54,** *106 (1946)*; *Nutrition Reviews* **4,** *237 (1946)*). The experimental subjects were divided into four groups; the first group was fed a palatable basal diet which was shown by analysis to provide 8 mg. of ascorbic acid daily, and the second group received the basal diet plus a supplement of raw cabbage providing 15 mg. of ascorbic acid daily (by analysis) so that their total intake was 23 mg. Group 3 received the same basal diet with the addition of 70 mg. of ascorbic acid daily, while the fourth group received the regular station diet, shown by analysis to provide 78 mg. of ascorbic acid daily.

Ascorbic acid was estimated in duplicate on (a) 10 cu.mm. serum samples (b) the

white cells obtained from 0.1 ml. blood, and (c) 0.0025 to 0.1 ml. aliquots of twenty-four hour urine specimens. The results showed that members of groups 1 and 2 (receiving respectively 8 and 23 mg. of ascorbic acid daily) had white cell values for ascorbic acid on the average only half as great as members of groups 3 and 4. For groups 1, 2, 3, and 4 respectively the mean white cell values were 11.9, 12.9, 24.2, and 23.7 mg. of ascorbic acid, while the mean serum levels were 0.18, 0.20, 0.79, and 0.73 mg. ascorbic acid per 100 ml. respectively. No serum values of zero were found and all but two samples contained 0.1 mg. per cent or more. There appeared to be a definite relation between the concentration in white cells and that in the serum. Among 39 subjects with serum levels above 0.4 per cent, only one had less than 20 mg. per cent in the white cells, whereas among 42 with less than 0.3 mg. per cent in the serum, only one had more than 20 mg. per cent in the white cells. In the intermediate zone (0.3 to 0.4 per cent in the serum) both low and high white cell levels were encountered.

At the end of eight months on the rigidly controlled ascorbic acid intakes 30 subjects, chiefly from group 1, were given large amounts of ascorbic acid and the course of their realimentation was studied. Each day for four days these subjects received 500, 1000, or 2000 mg. of ascorbic acid by mouth in eight or ten hourly doses. Twenty-four hour urine samples were collected and each morning, before any ascorbic acid was received, white cell and serum samples were collected. For the subjects of group 1, the serum and white cell levels were restored to normal in one day by a 2000 mg. dose, in two days by 1000 mg. doses each day, and in three or four days by 500 mg. each day. The serum and white cell responses of 6 subjects from group 2 were essentially the same, as might be expected since their white cell level before realimentation was little higher than for members of group 1. Four subjects from group 3 showed a further rise in the serum levels of ascorbic acid during realimentation, but their white blood cell levels did not rise above the relatively high levels caused by their 78 mg. daily intake.

Calculations were made of the daily retentions, based upon simple and admissible assumptions. From these it seemed reasonable to conclude that (1) with the ingestion of 8 to 23 mg. of ascorbic acid daily by adults the white blood cell ascorbic acid level is maintained at only about half the concentration attained with intakes of 78 mg. per day; (2) with 8 to 23 mg. daily of ascorbic acid a tissue deficit from the maximum possible concentration amounting to nearly 2 g. is observed; (3) with white cell values half of normal, serum ascorbic acid levels are found which are not necessarily zero, but which lie in the range 0.1 to 0.4 mg. per cent; (4) the white cell level appears to be an index of the total body level of ascorbic acid; and (5) under conditions supplying a sufficiently large amount of ascorbic acid the white cells and serum levels will return to normal values within twenty-four hours, with a retention of very large amounts of ascorbic acid in the body.

As is pointed out by the authors, an important question to public health is what tissue concentration is necessary for optimal ascorbic acid nutrition. The calculations suggested that among the control group of subjects receiving 78 mg. daily, about 400 mg. could be retained and the body content was 4 g. at saturation, so that they may be said to be 90 per cent saturated, while groups 1 and 2, receiving 8 and 23 mg. daily, respectively, were about 50 and 60 per cent saturated, respectively. Studies by Linghorne *et al.* (*loc. cit.*) in the primary investigation of the R. C. A. F. members suggested that groups 1 and 2 revealed a clearly defined handicap to the gingival tissue.

Comment is made by the authors upon the most desirable conditions for use in saturation tests for the assessment of

ascorbic acid nutrition. As they comment, it is usual to give a single fairly large dose, 200 to 500 mg. or even as much as a gram, and then to measure the amount of ascorbic acid which appears in the urine within a certain limit of time. But this quantity, particularly if given intravenously, is sufficient to flood the vascular system with ascorbic acid so that the amount excreted will depend on how well the kidney competes with other tissues of the body for the vitamin. When it is given by mouth, the serum level and therefore the excretion will depend partly on the rate of absorption. It must be concluded therefore that the information obtained from the test about the tissue deficit will be relative and not absolute.

The information obtained by the authors leads them to suggest an alternative procedure. The total dose given should be somewhat larger than the expected deficit, say 750 mg. to 1 g. for persons receiving over 50 mg. of ascorbic acid daily, or for persons suspected of serious depletion, 2 to 3 g. It should however be given by mouth in many divided doses through the day, and all the urine excreted in a twenty-four hour period, commencing with the first dose, should be collected. With allowance for an estimated 25 per cent destruction, the retention can be estimated, and an absolute figure thus determined. This procedure is more involved than that most commonly employed (which was of course chosen for its simplicity) but it has the advantage of giving an absolute measure of tissue depletion. It has already been applied by M. L. Hathaway and F. L. Meyer (*J. Nutrition* **21,** *503 (1941)*) in studies with children, using however much smaller doses given over a much longer period of time.

This study by the workers of the Public Health Research Institute of the City of New York sets on a much firmer foundation our knowledge of the interrelationship of serum or plasma levels of ascorbic acid with the levels of ascorbic acid in actively living tissues such as the white blood cells, and therefore by assumption with the levels in fixed tissues of the body.

FOLIC ACID AND SERUM ACETYLCHOLINE

In the present state of our knowledge of cellular metabolism, the discovery of a therapeutic effect of a vitamin or drug incites immediate interest as to the biochemical mechanism of such action. As is often the case, biochemical investigations of this type may lead only to correlative findings, but these in turn eventually lead to the detailed mechanism of the action of the substance concerned. The reports reviewed here are undoubtedly in the first stages of analysis of the action of folic acid, but in view of the recent discovery of the antianemic action of folic acid they are of special interest.

J. E. Davis (*Science* **104,** *37 (1946)*) indicates that folic acid may in some way be concerned with the level of cholinesterase in the blood serum, and in turn with serum acetylcholine activity. This investigator produced hyperchromic anemias in dogs by subcutaneous injection of 3 mg. of acetylcholine bromide twice daily for forty-seven days. While continuing the acetylcholine treatment, 2 of the dogs were given liver extract by daily injection, and one dog was injected daily with 2 mg. of folic acid. All of these animals responded by an increase in the percentage of reticulocytes and a regeneration of red blood cells to the normal level.

In similar experiments with dogs made anemic by feeding choline chloride, 2 dogs treated with folic acid and one with liver

extract showed reticulocyte rises to peaks of 3.4 to 4.2 per cent in six to nine days, and a normal erythrocyte count in twenty days. The serums of dogs made anemic in this way showed an "acetylcholine-like activity" after administration of 200 mg. of choline chloride, and this was less after treatment of the anemia. In one of the animals the serum cholinesterase activity, which was low during the period of anemia, was increased twelve times during liver therapy.

It might be supposed that this relation of cholinesterase to folic acid and liver extract would be restricted to acetylcholine or choline-produced anemias, but other experiments indicate a more general connection. Incubation of dog serums with folic acid or liver extract increased their cholinesterase activity as much as 93 per cent; one normal human serum was increased 15 per cent in the activity of this enzyme. Oral administration of 5 to 7.5 mg. of folic acid to 2 normal human subjects was reported to cause increases of 16 to 33 per cent respectively in their serum cholinesterase activity within five hours.

In a later report, J. E. Davis (*Science* **104,** *605 (1946)*) has indicated elevations in the acetylcholine content (bioassay) of serum of patients with pernicious anemia. Three pernicious anemia patients in relapse had serum acetylcholine levels of 15 to 18 micrograms per 100 ml., while 2 patients in remission had serum levels of 8 and 10 micrograms, and 2 normal subjects 6.5 micrograms per 100 ml. One of the patients in relapse was treated with 60 mg. of pteroylglutamic acid, another with 40 g. of ventriculin, and the third with 1 unit of liver extract daily. All 3 patients showed a decrease in serum acetylcholine of approximately 60 per cent as a result of the treatment.

The investigator's principal claim is that folic acid and liver extract act by increasing the formation of cholinesterase in the body. The data are not presented in detail in these short reports, but certainly they are not yet extensive. Assuming confirmation, these relations are of interest but difficult to interpret. Whether the anemic conditions and acetylcholine (and cholinesterase) levels are only coincidental or directly and intimately concerned through pteroylglutamic acid, is not yet evident. Nevertheless this suggestion may develop into a better understanding of the biochemical changes in anemia and the role of folic acid.

NUTRITIONAL REQUIREMENTS OF ANIMAL TISSUES GROWN *IN VITRO*

Since the pioneer work of R. G. Harrison (*Proc. Soc. Exp. Biol. Med.* **4,** *140 (1907)*), M. T. Burrows (*J. Am. Med. Assn.* **55,** *2057 (1910)*), and A. Carrel (*J. Exp. Med.* **17,** *14 (1913)*) it has been known that animal cells could be grown successfully away from the intact animal. If such tissue cultures are supplied with the appropriate nutrient substrates, frequently renewed, they continue to grow, and by frequent subculture under sterile conditions can be maintained for years in a viable and growing condition (e.g. A. H. Ebeling, *Ibid.* **35,** *755 (1922)*).

It is apparent that such sterile cultures of isolated tissues offer special opportunities for determining the nutritional requirements of the animal organism. Not only could the gross nutritional requirements be determined with such cultures, but the effects of over- and undersupply of specific nutrients on "normal" and "abnormal" growth processes might be determined.

That greater use of tissue cultures in this connection has not been made reflects the difficulty of the problem rather than the novelty of the idea. For successful, long-continued culture, a nutrient medium con-

taining clotted plasma and embryo juice has become standard (Carrel, *loc. cit.*), and the few attempts which have been made to substitute materials of known constitution for these complex biologic fluids have met with uniform failure.

The problem has been approached anew by P. R. White (*Growth* **10,** *231 (1946)*), who formulates the long-term objective of his study to be "the development of nutrients all of whose components are precisely known both as to quantity and as to chemical and physicochemical composition, which shall be capable of supporting potentially unlimited survival and/or growth of the tissues in question." Skeletal fibroblasts, epithelium, and skeletal and heart muscle from 8-day chick embryos were chosen for investigation. These were cultivated in roller tubes similar to those described by G. O. Gey (*Am. J. Cancer* **17,** *752 (1933)*). The nutrient medium was wholly of known composition, compounded to be as complete as present knowledge would permit. It contained inorganic salts, glucose, the ten essential amino acids, all of the known B-vitamins, ascorbic acid, vitamin A, glutathione, and cysteine. The ratios of the various components is of great importance; preliminary experiments established the suitability of concentrations used for each of the major groups of materials present in the medium. The old nutrient medium was removed and replaced by fresh medium every few days.

In this exploratory study, growth and well-being were followed qualitatively by observation of cell migration and survival time as these were affected by variations in the nutrient medium. With heart muscle, heart beat became evident in the explant within a few hours after excision and transfer to the nutrient medium. Growth of both muscle and fibroblasts took place. Muscle strands became recognizable at about the third day. These gradually extended until broad, strap-shaped bands and anastomosing nets were set up. The "heart beat," at first confined to the original explant, became established in these outer bands as well after six to eight days. One culture showed strong rhythmic contractions for twenty-eight days, when it became contaminated. Another continued to beat for forty-four days through five renewals of nutrient. Fibroblasts in these same cultures underwent progressive degeneration after the first ten days, but muscle bands reached their maximum development only after two or three weeks and thereafter showed little regression or degeneration not directly traceable to injury of some sort.

Encouraging results were also obtained with other tissues. Skeletal fibroblasts were maintained in good condition for as long as fifty-eight days; epithelium and nerve tissues were grown for shorter periods. In purely inorganic nutrient solutions, chick cells seldom survive longer than four or five days. The results are thus "sufficiently encouraging to lend support to the conviction that potentially unlimited growth of animal tissues, *in vitro*, can be obtained in a fully synthetic medium and a true science of tissue nutrition can be based thereon." It is recognized, however, that the present medium is far removed from this ultimate goal. Perhaps for this reason, the effect of deletion of individual components of the medium on growth and survival of the tissues has not been determined. However, it was shown that the presence of the amino acids is of prime importance for growth and survival, thus confirming the independent work of A. Fischer (*Acta Physiol. Scandinav.* **2,** *145 (1941)*); the role of individual amino acids, vitamins, and trace elements under these conditions remains for future work to establish.

NOTES

Letter to the Editor

Dear Sir:

A recent letter by E. W. McHenry (*Nutrition Reviews* **5,** *95 (1947)*) referring to an earlier paper of ours (DeW. Stetten and G. E. Boxer, *J. Biol. Chem.* **153,** *607 (1944)*) calls for comments and corrections. In our paper, McHenry to the contrary notwithstanding, specific reference was made to earlier isocaloric feeding experiments as well as to the work of Whipple and Church, and to that of McHenry. McHenry states, "The pair group given thiamine was 10 to 11 g. lighter at the start." The source of this statement is unclear, as these figures were not included in the published paper. In actual fact, as could have been inferred from the growth curves and the final weights, which were given, the mean weight of the rats in this group was 41.5 g. initially, those of the other groups 40 and 41 g. McHenry's comment (paragraph 2c) implies that the quantity of fat synthesized is a function of the deuterium concentration alone. This is not the case. A brief study of the arithmetic included in our paper will reveal that the estimated quantity of fat synthesized is a function of the product of the deuterium concentration and the quantity of fat present.

In summary of our experiments, three groups of rats were compared with respect to composition and to rate of fatty acid synthesis. One group was fed a complete diet ad libitum, another was offered the same diet ad libitum with thiamine excluded, and a third group received the complete diet limited in quantity to that consumed by the second group. No significant differences between the latter two groups were demonstrable in regard to growth rate, fat content, or rate of synthesis of fatty acids. This synthetic rate was, in both of these latter groups, far below that of the normally growing rats. "These results indicate that the loss in fat, whether the result of thiamine deficiency or of restricted food intake, is referable to a very marked retardation of deposition of newly synthesized fatty acids. . . . They show, furthermore, quantitative and qualitative similarity in the processes that resulted in the loss of fat in the thiamine-deficient rats on the one hand and in the rats on restricted rations on the other hand."

It is our view that for the deposition of newly synthesized fat to proceed at optimal rate in the growing animal a number of conditions must be met, among these, adequate caloric supply and an adequate supplement of thiamine, or, as stated in our paper, ". . . a supply of thiamine is a necessary but not sufficient condition." Whereas there may be experimental conditions in which a limitation upon the rate of fatty acid synthesis is set by lack of the specific enzymes in which thiamine participates, there was every reason to believe that in our thiamine-deficient rats, this limitation was set by the diminished food intake.

In conclusion may we state that we have at no time subscribed to the point of view apparently attributed to us by McHenry, that thiamine is unnecessary for the synthesis of fat from carbohydrate.

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Calories and Work Output

H. A. Kraut and E. A. Muller of the

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Kaiser-Wilhelm Institute of Physiology (*Science* **104,** *495* (*1946*)) have reported war-conducted studies on the subject of calories and work output. As the authors state, the war situation "induced a nutrition experiment of the widest range." Lack of calories had to be met by controlled rationing. A decreased ration to the laborer inevitably led either to a decreased work output or to a loss of body weight with temporarily maintained output. The German investigators divided their consideration of calories into those required for metabolism and for digestion during rest, termed the basal metabolism calories, and amounting to about 1600 calories, and those needed for muscular activity, termed the work calories.

In their first study, in 1942, 20 workmen were observed who were engaged in dumping debris and changing rails in the construction of an embankment. These men were fed by the factory and their work output could be calculated by the number of tons of debris dumped per hour per man. For several months the men had been receiving 820 work calories per day and dumped 1.5 tons per hour. Without informing either the workers or their supervisors, the rations were increased to provide 1300 work calories. During a year on this program, the work output increased to 2.2 tons per man, and since the men gained an average of 4 kg. in weight, it was presumed that even this caloric increase was not fully utilized for work. The workers were promised a reward of cigarettes for an increased minimum production, first of 2.5 tons, and later to 3 and even 4 tons. The output during the six month period of rewards finally reached 3.4 tons per man, but a steady loss of body weight occurred, amounting to about 3.5 kg. per man.

Careful records were kept of coal output and food rations in the Ruhr from September 1939 to September 1944. The cut in rations during this period resulted in a steady decrease in coal production. Although work output per man could be maintained temporarily despite a sharp cut in rations, this was done at the expense of body weight.

In general it is concluded that a worker tends to balance his output of work with caloric intake, and to preserve his body weight. Special incentives may upset this relation, producing an extra output of work, but this is usually temporary and inevitably makes demand on the body substance. The latter event, if continued, leads only to decreased capacity and finally to work failure. Under conditions of acute food shortage, industrial management reverts largely to those responsible for food administration. The speed of reconstruction in Europe during the present period will no doubt be strongly affected by the caloric supply.

Nutrition Bulletin

The Central Council for Health Education, Tavistock House, Tavistock Square, London, W. C. 1 issued in January, 1947 the first number of a publication known as the Nutrition Bulletin. This is intended for those who are interested in the application of nutritional knowledge in all the fields of social welfare and public health. The articles are concise, topical, and written in nontechnical language.

The bulletin is to be issued every two months at a subscription price of 3 shillings.

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